Developmental Neurotoxicity Testing of Nano-particle in Neurosphere Assays Using Human Neuronal Progenitor Cells

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（神経前駆細胞を用いた神経塊アッセイにおけるナノ粒子の発生神経毒性に関する研究）

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Abstract of thesis

In this research, the author established a stable and efficient neurospheres culture system for developmental neurotoxicity (DNT) testing using Human Neuronal Progenitor Cells (hNPCs), and analyzed the DNT of nano-particle in neurosphere assays using the neurosphere culture system. The dissertation is divided into 4 chapters.

In chapter 1, the author introduced the background of this research, including PAMAM dendrimer nanoparticles, developmental neurotoxicity, human neural progenitor cells and three-dimensional neurosphere culture system. It was pointed out that engineered nanomaterials or nanoparticles have been promoted in the practical application of medical devises or industrial utilization but the neurotoxicity of nanomaterials in human body remains unclear. The objective of this research was proposed in this chapter to evaluate DNT of engineered nanomaterials, a three-dimensional neurosphere culture system developed based on hNPCs.

In chapter 2, the author established the process for a stable and efficient neurospheres culture system for DNT testing. This chapter described the importance of basement membrane during neuronal differentiation, and compared the effects of four kinds of basement membrane on cell differentiation. The results showed that cells cultured on the LM511 matrix exhibited increased neurite lengths compared to those cultured on Laminin 111 (LM111). This indicates that LM511 is an appropriate and stable matrix for neurosphere culturing, and that integration of lamina-dense structure provides an ideal surface for neurite outgrowth. The author suggested that these extracellular microenvironments are believed to provide binding sites for neural progenitor cells, activating intracellular signaling pathways and promoting sphere formation and neurite differentiation. This chapter also tested the DNT of Benzo[a]pyrene and 5-Azacytidine to verify whether the established system can be used. The results showed that both chemicals significantly inhibited cell migration and induced apoptosis, suggesting that this system can be used to test DNT.

In chapter 3, the author assessed DNT of PAMAM dendrimer nanoparticles using the established culture system, and DNT was investigated with morphologic approach. The author investigated biodistribution using fluorescence-labeled nanomaterials, which was observed by confocal microscopy, including proliferation, migration, differentiation, and apoptosis, a basal process during brain development. This chapter also evaluated gene expression using microarray analysis followed by pathway and network analysis. The results from 3 days' exposure to PAMAM dendrimer-NH2 located in the center of neurosphere suggested that PAMAM can penetrate into the center of neurospheres from the superficial cells. A reduced number of MAP2-positive cells but not neurite length/cells was

noticed after PAMAM -HN2 exposure, indicating an inhibitory effect on neuronal migration but not differentiation. The author confirmed the cell proliferation assay, suggesting that PAMAM-NH2 inhibited cell proliferation and migration in a dose-dependent manner. The author also found that PAMAM-SC (sodium carboxylate) did not affect neurospheres at any tested concentration, implying that naturally charged PAMAM-SC would be a candidate of drug carrier. The network analysis showed that there are three connected networks of the selected gene targets in direct interactions, network targets and regulators, adipogenesis, myometrial relaxation/contraction, and insulin-like growth factor signaling. The results also indicated the key genes involved in PAMAM-NH2 exposure, which were confirmed by RT-PCR method. Furthermore, results from this research suggested that the exposure of neurosphere to PAMAM-NH2 dendrimers leads to a change in cell proliferation, and cell migrations through *TFPI2*, *IGFBP3*, *AMD* and *EGR1*.

Finally, in the chapter 4, the author summarizes the conclusions of this research. A stable and efficient culture system for neurotoxicity assessment was established to distinguish between neurotoxic and non-neurotoxic substances. Using this novel culture and assessment system, the author found that naturally charged PAMAM-SC will be a good candidate of drug carrier. PAMAM-NH2 can penetrate into cells and neurospheres depending on time, with resultant change in cell proliferation and cell migration, use of PAMAM-NH2 as drug carrier may induce adverse reactions on neurons. It is believed that this system will be a novel method to assess DNT of nanoparticles and chemical substances.

Abstract of assessment result

This study analysis the developmental neurotoxicity (DNT) of nanoparticle in neurosphere assays using human neuronal progenitor cells (hNPCs). A stable and efficient neurospheres culture system for DNT testing using hNPCs was established. Also, DNT of PAMAM dendrimer nanoparticles was assessed using the established culture system and DNT was investigated by morphologic approach. The results showed a reduced number of MAP2-positive cells but not neurite length/cells after PAMAM -HN2 exposure, indicating an inhibitory effect on neuronal migration but not differentiation. The results were confirmed by cell proliferation assay, suggesting that PAMAM-NH2 inhibited cell proliferation and migration in a dose-dependent manner, and use of PAMAM-NH2 as drug carrier may induce adverse reactions on neurons. This study demonstrated the key genes for PAMAM-NH2 exposure, which were confirmed by RT-PCR method. The author also found that PAMAM-SC (sodium carboxylate) did not affect neurospheres at any tested concentration, suggesting that naturally charged PAMAM-SC would be a good candidate of drug carrier. In conclusion, the novel culture system can efficiently and stably be established, which is a good tool for investigating the roles of neurotoxins or therapeutic agents in neuronal disease. The promising novel culture system established in this research is believed to have contribution to the DNT assessment of nanomaterials and therapeutic agents in the future.

The final examination committee conducted a meeting as a final examination on 1st August, 2017. The applicant provided an overview of dissertation, addressed questions and comments raised during Q&A session. All of the committee members reached a final decision that the applicant has passed the final examination. Therefore, the final examination committee approved that the applicant is qualified to be awarded the degree of Doctor of Philosophy in Biotechnology.